THE EFFECTS OF REPEATED MORPHINE EXPOSURE ON METABOTROPIC GLUTAMATE RECEPTOR ACTIVITY IN ADOLESCENT MICE

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

The Effects of Repeated Morphine Exposure on Metabotropic Glutamate Receptor Activity in Adolescent Mice. (May 2013)

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Adolescent mice exhibit marked changes in D2/D3 dopamine receptor activity following administration of certain opioids. Thus, the present study examined whether repeated exposure to morphine would also modulate the activity of metabotropic glutamate receptors (mGluRs) in the dorsal striatum of adolescents. Administration of a group I-specific mGluR agonist, S-3,5-dihydroxyphenylglycine (DHPG), induces face-washing behavior in adolescent mice. Since this behavioral effect has been shown to be mediated by mGluR1, this behavior was used as an indicator of mGluR1 activity in the dorsal striatum. Adolescent mice (n= 9-13 per group) were first examined for their behavioral response to unilateral administration of DHPG directly into the dorsal striatum. Morphine (20 mg/kg, s.c.) was then administered once daily for 6 days. The response to DHPG was re-examined at one of three times, i.e., 2, 4, or 24 hours following administration of the final dose of morphine. At 2 hours following administration of the last morphine dose, mice showed a significant decrease in response to DHPG (i.e. duration of face-washing behavior) relative to their pre-morphine response. At 4 and 24 hours following administration of the last morphine dose, no difference in response to DHPG administration was

observed relative to their response prior to morphine administration. This suggests that repeated morphine administration causes a decrease in mGluR1 activity in the dorsal striatum of adolescent mice, which could affect long-term neural activity. Future studies will examine long-term effects of repeated morphine exposure in adolescent mice, as well as the possibility of differential effects by various opioids. Additionally, the effects of repeated morphine exposure on mGluR signaling should be also examined in adults.

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First, I would like to thank my advisor and mentor, Dr. Shoshana Eitan, for her unending guidance and patience. Shoshy, thank you for your continuing support. Without it, I would not have had the opportunity—much less the ability—to achieve as much as I have.

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NOMENCLATURE

Ad libitum	Free access (to food and water)
Agonist	A chemical which binds to a receptor and activates it
Alexa 488	A florescence marker used to detect cannula placement site (and in our experiment tagged to donkey anti-rabbit IgG antibodies)
Bregma	Anterior landmark on the skull
C57BL/6	Inbred strain of laboratory mouse (C57 Black 6)
c-fos	A transcription factor used as an indirect marker of neural activity
Cannula	A tube implanted into a specific region of the brain, to allow for repeated administration of drugs into the same location
DA	Dopamine, a neurotransmitter
D2/D3	Inhibitory dopamine receptors
DHPG	(S)-3,5-dihidroxyphenylglycine, a Group I mGluR agonist
DMSO	Dimethyl sulfoxide, a powerful organic solvent
Dorsal Striatum	Brain area that receives input from the cerebral cortex, known to be involved in movement and addiction

FST	Forced Swim Test, used to measure depressive-like behaviors in rodents
Group I mGluR	Category of receptors including mGluR1 and mGluR5
ICV	Intracerebroventricular, an injection into the ventricles of the brain
Intraperitoneal injection	Injection into the abdominal cavity, IP
Lambda	Posterior landmark on the skull
mGluR	Metabotropic glutamate receptor, a relatively slow-acting receptor for glutamate that acts via a G protein
µL	Microliter, 1×10^{-6} liter
nmol	Nanomole, 10^{-9} moles, a measure of the mass of a chemical in solution
PND	Postnatal day, a common method of indicating the age of a rodent
Subcutaneous injection	Injection beneath the surface of the skin (s.c.)

CHAPTER I

INTRODUCTION

Adults and adolescents differ in their sensitivity to drug exposure, which is cause for concern due to the increased vulnerability of adolescents to drugs of abuse. For example, it has been shown that adolescent rodents show less locomotor sensitization and amphetamine-induced stereotypies than adults (Bolanos et al., 1998; Lanier and Isaacson, 1977; Laviola et al., 1999; Laviola et al., 1995; McKinzie et al., 1999; Snyder et al., 1998; Spear and Brick, 1979), and that adolescent rats and mice show greater sensitization to the hyperlocomotion seen following morphine administration than their adult counterparts (Hofford et al., 2012; Spear et al., 1982). Although there are few studies of this sort on humans, the available data suggests that cocaine is more addictive in adolescent than adult humans (Estroff et al., 1989), and that adolescents may receive less pleasure from use of some drugs than adults. Rapoport et al. (1980) reported that prepubescent males felt no euphoria following a single administration of either a high or low dose of dextroamphetamine, while adult males reported euphoria at both doses of the drug. This increases the likelihood that adolescents will use more of a drug of abuse than adults will, in order to feel the same level of positive effects (such as euphoria) as adults. Finally, drug use beginning in adolescence increases the reported incidence of co-morbidity with psychiatric disorders relative to drug use beginning in adulthood (Kaminer and Bukstein, 2008), and adolescent substance use is a strong predictor of substance abuse later in life (Deyken et al., 1987; Fergusson et al., 1994; Friedman and Humphrey, 1985; Grant and Dawson, 1997; Hawkins et al., 1997; Prescott and Kendler, 1999; Rachal et al., 1982) (for review, see Spear, 2000).

Similarly, age differences are seen in reactions to opioid withdrawal in mice, with adolescents showing less immobility than adults in the Forced Swim Test (FST), suggesting that they experience less negative mood during morphine withdrawal than their adult counterparts. Additionally, these data could suggest that different patterns of changes in opioid receptor activity occur following morphine exposure or withdrawal in adolescents as compared to adults, as different opioid receptors have previously been shown to modulate FST behaviors (Hodgson et al., 2009, Hodgson et al., 2010). Additionally, our lab has previously found a difference in the functionality of the D2/D3 dopamine receptors between adolescent and adult mice following repeated morphine exposure, with adolescents showing a greater increase in postsynaptic D2/D3 receptor activity than adults (Hofford et al., 2012). Similar results were found following repeated methadone—but not buprenorphine—exposure, and the conclusion was drawn that buprenorphine might be safer than methadone as an opioid maintenance treatment in adolescents because it does not cause this neurochemical change, which may have long-term mental health implications (Barwatt et al., 2012).

The present study explored the effects of repeated opioid exposure on the metabotropic glutamate receptors (mGluRs) in the dorsal striatum of adolescent mice. As reported by Tsapakis and Travis (2002), glutamate has been shown to play a role in schizophrenia, as well as affective disorders. An understanding of alterations in the functionality of mGluRs in adolescents following repeated morphine exposure would aid in revealing the role of the glutamatergic system in addiction and these other psychiatric disorders, particularly in regard to the specific vulnerabilities of adolescents. Thus, this study aimed to determine to what extent exposure to drugs of abuse, particularly opioids, alters the functionality of the glutamate system in this age group. This might

offer an explanation as to why adolescents are particularly vulnerable to the risks of drug use and abuse (Spear, 2000), and could be used to aid in development of prediction and treatment plans for opiate addictions in adolescents.

Eight mGluRs have been identified to date, and we specifically studied Group I mGluRs (mGluR1 and mGluR5), which have previously been shown to mediate drug-induced behavioral effects in adult rats (Gass et al., 2009). Additionally, Mao and Wang (2002) showed that the increase in c-fos expression in the rat striatum following acute amphetamine administration (seen by Cole et al., 1992, Graybiel et al., 1990, Moratalla et al., 1992, Nguyen et al., 1992) is mediated by Group I mGluRs.

In order to measure the functionality of Group I mGluRs, we examined the behavioral response of mice to a Group I-specific mGluR agonist. It has been shown that injection of the agonist, (S)-3,5-dihidroxyphenylglycine (DHPG), into the dorsal striatum augments behavioral activity level in rats (see eight-point scale in Mao and Wang, 2000, Wang and Mao, 2000) and induces contralateral rotation and hyperlocomotion, as well as stereotyped behaviors (Mao and Wang, 2000, Wang and Mao, 2000). In mice, a similar injection (administered intracerebroventricularly, or ICV) at low doses leads to increased occurrence of grooming behaviors, specifically face-washing and scratching of the face (Barton and Shannon, 2005), which persists for at least 30 minutes following the injection (Hikichi et al., 2008). In both rats and mice, seizures are seen following administration of high doses of DHPG, and sometimes result in death (Mao and Wang, 2000, Hikichi et al., 2008). Hikichi et al. (2008) concluded that face-washing activity following DHPG administration is a

useful behavioral readout of mGluR1 activity, which is why this behavior was chosen to assay in the present study.

Given that, this study examined the behavioral response to low doses of DHPG administered intrastriatally in adolescent mice following repeated exposure to morphine relative to the behavioral response in these same mice prior to morphine exposure. Observation of face-washing behavior was used in order to understand how the morphine exposure affects the behavioral response to the agonist.

Changes in behavioral response would suggest molecular changes in mGluR1 activation in the dorsal striatum. We aimed to identify and characterize both the behavioral and the molecular changes in an adolescent mouse model of repeated opioid use. Later work will examine this effect in adults so that age differences can be examined. The dorsal striatum was chosen as the injection site in part due to its potential role in the habitual aspects of drug seeking (Vanderschuren et al., 2005), and in part due to evidence that exposure to certain drugs of abuse cause changes in glutamatergic function in the dorsal striatum of rats (Mao and Wang, 2002, Arai et al., 1996), which then lead to increases in opioid peptide gene expression (Mao et al., 2008).

In this study, guide cannulae were implanted in the dorsal striatum of adolescent male mice. Following a behavioral pretest, the mice were given a six-day morphine regimen, with subcutaneous injections of 20mg/kg morphine (10ml/kg) at 09:00 each day. Two, 4, or 24 hours following the last morphine injection, a posttest was conducted by injection of DHPG into the

guide cannulae and the animals were then observed for differences in face-washing behaviors relative to their drug-naïve recordings.

We expected repeated morphine administration to cause a change in DHPG-induced face-washing behavior, indicating a change in mGluR1 activity in the dorsal striatum caused by morphine exposure.

CHAPTER II

METHODS

All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Texas A&M Institutional Animal Care and Use Committee.

Animals

Adolescent male C57BL/6 mice (n = 30) were purchased from Harlan Lab, Houston, TX at postnatal day (PND) 22 and were group housed, with 2 - 3 animals per cage. The colony room was maintained at 21 ± 2° C and 50 ± 5% humidity and followed a 12h/12h light/dark cycle (lights on at 07:30). Animals were given at least 4 days to acclimate to the colony room following arrival, with food and water ad libitum. Cannulation surgeries were performed on PND 27 – 28, when animals were 10 – 13 g. The first behavioral observation (pre-morphine DHPG challenge) occurred between PND 33 and 35, with the second behavioral observation (post-morphine DHPG challenge) occurring 7 - 10 days later. The amphetamine challenge occurred several days after the post-morphine DHPG challenge. See Figure 1 for a complete timeline.

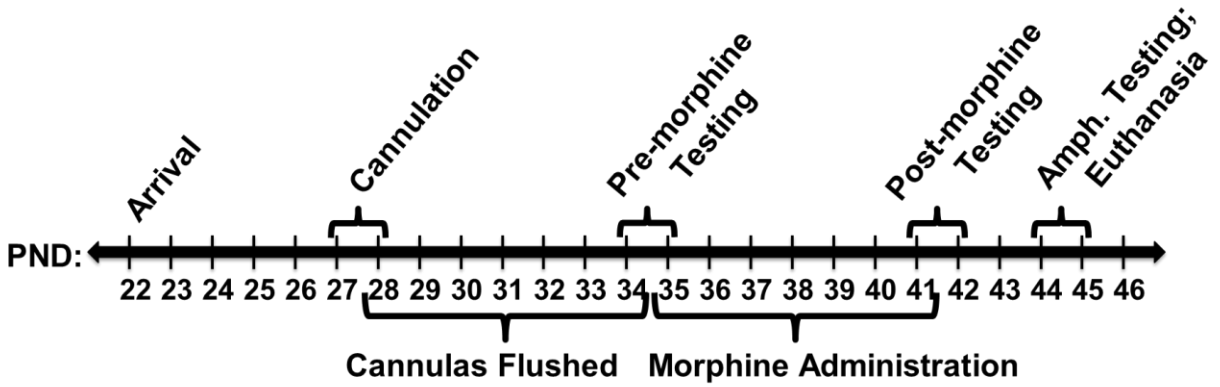

Figure 1 Timeline This diagram shows the overall experimental timeline, from arrival of animals to euthanasia.

Stereotaxic Surgeries

Animals were anesthetized with intraperitoneal (IP) injections of 100 mg/kg ketamine and 10 mg/kg xylazine (mouse cocktail) before being mounted on a David Kopf stereotaxic holder adapted for mice. Booster doses of .02-.05 mg ketamine and .002-.005 mg xylazine were injected IP as necessary throughout the surgeries. A screw was inserted on the left side of the skull, approximately 1 mm anterior to lambda and -2 to -3 mm lateral of the midline to anchor the hardware to the skull (individual placement varied due to skull and incision size). A 23 gauge stainless steel cannula 8 mm in length was placed in the central portion of the dorsal striatum at a depth of 2 mm (0.45- 0.65 mm anterior to bregma and 1.10-1.50 mm lateral of the midline, depending on skull size, with placement usually around 0.50 mm anterior to bregma and 1.20 mm lateral of the midline) unilaterally, on the right side. Hardware was secured by applying a layer of super glue, followed by a layer of dental cement, and finally another layer of super glue following placement of threads on the cannula. Cannulae were sealed with a stainless steel insert made of 30 gauge wire, and then covered with a screw-on plastic cap. Animals were given an 10mL/kg IP injection of 0.4% enrofloxacin (Baytril) to prevent post-surgical infection prior to placement in a recovery cage. As we are studying the effects of opioid use, administration of analgesics would invalidate any results obtained, and thus no analgesics were administered.

Following cannulation, animals were observed and kept warm until they had regained consciousness. Once animals were able to walk around the recovery cage, they were returned to the home cage and provided with softened food. For 10 days following surgery, water in the home cage was infused with 1mL 10% enrofloxacin per 200mL water for continuous antibiotic

administration. Cannulae were flushed once daily with 1 µL of 0.9% saline solution ("saline") for the six days following cannulation (using the same method as for the DHPG microinjections described below). Both the insert and the injector protruded 1 mm past the cannula, into the dorsal striatum. The pre-morphine DHPG challenge was carried out no earlier than the last day of saline flushes.

Drugs

Morphine

Morphine sulfate was purchased from Sigma (St. Louis, MO), diluted to 80 mg/kg (8 mg/mL), kept at 4°C and further diluted to 20 mg/kg prior to administration.

Administration

Animals were injected with 20 mg/kg of morphine subcutaneously once daily (09:00) for six days, for an injection volume of 10 mL/kg (per injection).

Saline controls have not been included so far due to limited time, so as to increase the n for all groups, but will be run in the near future.

DHPG

5 mg of (*S*)-3,5-dihydroxyphenylglycine hydrate was purchased from Sigma Aldrich. This was dissolved in 1 part DMSO and 9 parts saline, to a concentration of 160 nmol/µL. This was divided into 5 µL aliquots and stored at -20° C. Individual aliquots were diluted to 10 nmol/µL using additional saline prior to being administered.

Administration

Each animal was removed from its home cage and placed on the wire rack of a foreign cage, at which point the cap and insert protecting the cannula were removed. In place of the insert, a 9 mm length of 30 gauge hypodermic tubing ("injector") attached to a length of PE-10 tubing ("tubing") was placed inside the cannula. This tubing was attached to a 10 µL Hamilton microsyringe, placed in a World Precision Instruments microsyringe pump controller (Micro4). The tubing was filled with saline before 1 µL of 10 nmol/µL DHPG was withdrawn into the tubing. This DHPG was then infused at a rate of approximately 0.25 µL/min into the cannula while the animal moved freely on the rack of the injection cage. The progress of the injection was monitored by observing the movement of a small air bubble through the precalibrated tubing. Following the 1 µL injection, the injector and tubing were left in place for at least 1 minute to prevent any backflow of the solution along the injection track. The injector was then removed, the insert and cap were replaced, and the animal was placed back into the testing chamber, as described below.

Vehicle controls have not been included so far due to limited time, so as to increase the n for all groups, but will be run in the near future.

Behavioral Observations

On each test day, animals were given 30 minutes to habituate to the behavior room, which had dim lighting and a 40dB white-noise machine. Following habituation, animals were placed individually (one animal per apparatus) into plexiglass cylinders 15 cm in diameter and 40 cm tall for observation, with 2 to 4 animals being tested simultaneously, physically and visually separated from each other. Animals were video-recorded for 30 minutes. Following the baseline recording, all animals were returned to their home cage for several minutes before being removed individually for DHPG microinjections as described above, prior to being replaced in the same plexiglass cylinder for a post-injection recording of 60 minutes. Note that the cylinders were cleaned with ethanol and allowed to dry completely between all recordings.

Animals were tested on or after the sixth day following cannulation, prior to any morphine treatment. Animals were then treated with morphine for six days and tested again 2, 4, or 24 hours following the final injection (11:00 or 13:00 on day six or 09:00 on day seven of the injection regimen).

Cannula Placement Verification

Assessment of cannula placement, by microscopy analysis of the anatomical location of a fluorescence marker, has not been performed yet, but an attempt was made to behaviorally make a preliminary determination of placement accuracy. One to three days following the final behavioral observation, animals were microinjected with 80 nmol of d-amphetamine in 1 µL of saline and video-recorded to observe their behavioral response. Since the cannula was targeted to the dorsal striatum, it was hypothesized that a unilateral injection of d-amphetamine would cause rotation primarily in a counter-clockwise direction if the cannula were correctly placed, as locomotion should be inhibited on the left side of the animal following excitation of the dorsal striatum. Videos were scored, and any animals that did not show an obviously positive response were tested again the following day, to rule out the possibility of unsuccessful completion of the microinjection. For the few animals that did not show a counter-clockwise rotational bias following the second test, a final test was performed using double the d-amphetamine dose. Data from any remaining animals was not used.

Following the final d-amphetamine testing period, animals were microinjected with 1µL of Alexa 488 donkey anti-rabbit IgG to allow for visualization of cannulation placement at a later date. Animals were then deeply anesthetized with 0.1-0.25 mL of 5 mg/mL pentobarbital prior to being decapitated. Brains were extracted and were flash-frozen in Tissue Tek OCT compound using acetone and dry ice before being stored at -80° C.

Behavioral Scoring

Face-washing behavior was defined as using the forepaws to wipe from the ears to the mouth. Three or more blind observers manually scored each video for this behavior. Sportline 220 Sport Timers were used to record the duration of face-washing behavior during each 1 minute bin following the microinjection. The last 5 minutes of the 30 minute baseline period were scored, as were the first 5 minutes following DHPG or vehicle administration. Scores were compiled by the author.

Data Analysis

The 1 minute bins for each recording were combined, and scores from individual observers averaged, so that each animal had a total of 4 scores: pre-morphine baseline, pre-morphine post-DHPG injection, post-morphine baseline, and post-morphine post-DHPG injection. All scores represented the total duration of face-washing behavior in seconds for the 5 minute bin.

Additionally, the Δface-washing (post-DHPG injection – baseline) was calculated for each test day (pre-morphine and post-morphine) and defined as the DHPG effect. Finally, the ΔDHPG effect was calculated for each animal, by subtracting the pre-morphine DHPG effect from the post-morphine DHPG effect.

Once the data was split into groups by post-morphine test time (2, 4, and 24 hours), scores of individual animals were averaged and were compared between groups. The pre-morphine face-washing scores were also compared independent of group, to determine the DHPG effect on drug-naïve animals.

CHAPTER III

RESULTS

Pre-Morphine DHPG Challenge

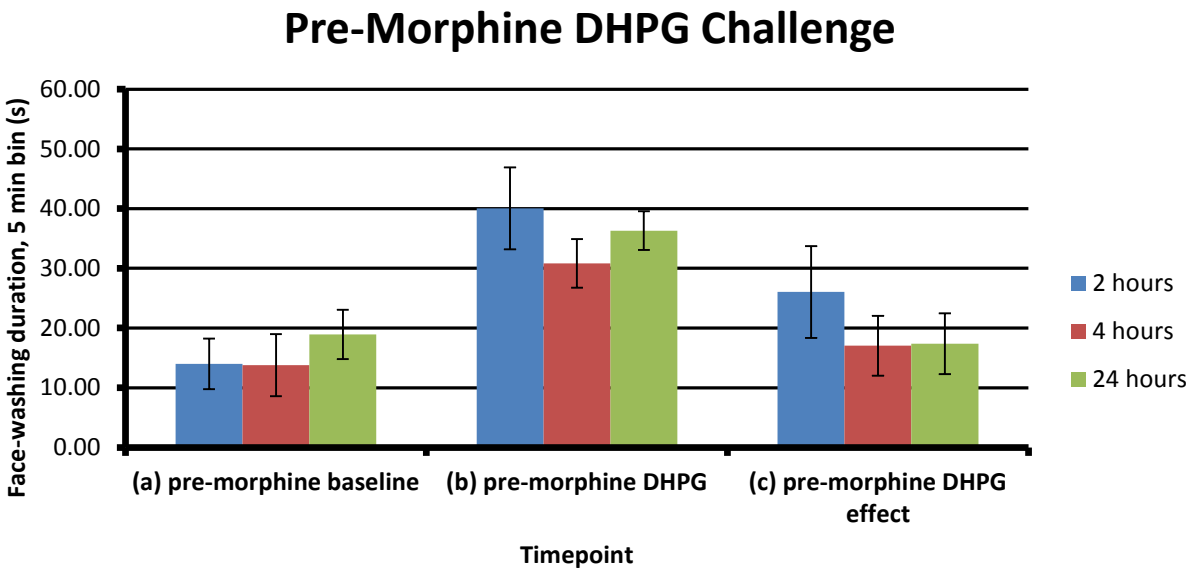

Figure 2 Pre-morphine DHPG challenge This figure shows the average duration of face-washing behavior, in seconds, during the final five minutes of the baseline recording (a) and first five minutes of the post-DHPG injection recording (b), by group prior to morphine administration. (c) shows the average pre-morphine DHPG effect by group, defined as the difference between (b) and (a). No significant differences were found between groups at any of the three timepoints. All results are presented as mean ± SEM.

Baseline Face-Washing Activity

The average face-washing behavior duration (in seconds) during the final 5 minutes of baseline recording in drug-naïve animals is presented for each experimental group in Figure 2a. One-way Analysis of Variance (ANOVA) showed no significant differences between the experimental groups prior to the exposure to morphine or DHPG ($F(2,27) = 0.422$, $p = 0.660$).

DHPG-Induced Face-Washing Activity

The average face-washing behavior duration (in seconds) in drug-naïve animals in the first 5 minutes following intrastriatal injection of 10 nmol DHPG is presented for each experimental group in Figure 2b. One-way ANOVA showed no significant differences between the experimental groups in response to DHPG administration prior to the exposure to morphine ($F(2,27) = 0.733$, $p = 0.490$).

DHPG Effect

DHPG effect was defined as the difference in face-washing behavior duration (in seconds) between the post-DHPG injection and the baseline timepoint recordings. DHPG significantly increased face-washing behavior in the drug naïve (pre-morphine administration) adolescent mice (Figure 3; $t(29) = 5.596$, $p < 0.001$). Importantly, one-way ANOVA revealed no significant differences between the different experimental groups prior to the exposure to morphine (Figure 2c; $F(2,27) = 0.626$, $p = 0.542$).

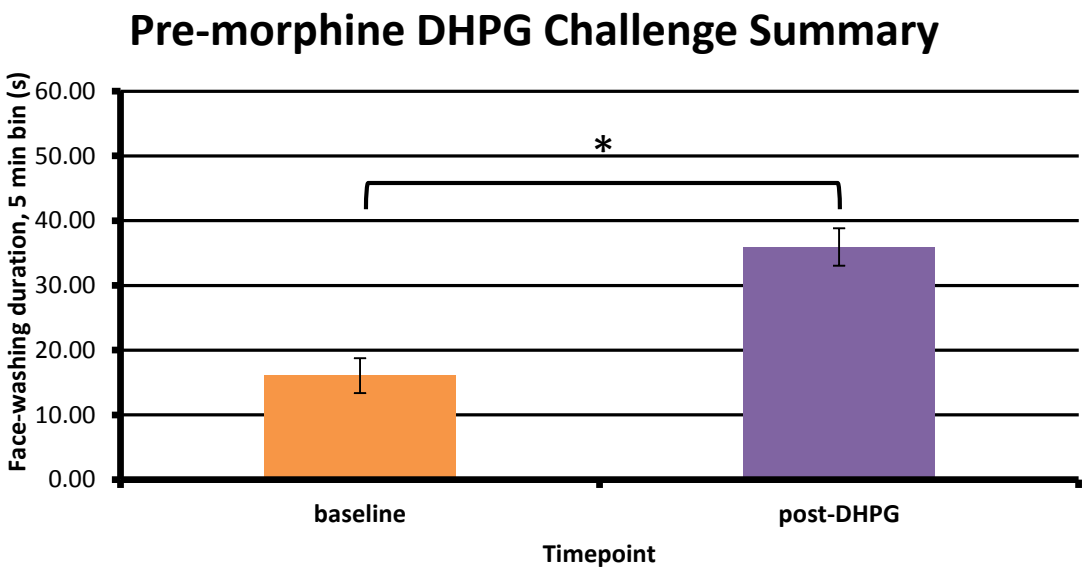

Figure 3 Pre-morphine DHPG challenge summary This figure shows the average duration of face-washing behavior, in seconds, during the final five minutes of the baseline recording and first five minutes of the post-DHPG injection recording across all groups prior to morphine administration. The average duration of face-washing behavior following DHPG injection was significantly higher than that seen during the baseline recording, independent of group. (*) indicates a significant difference ($p < 0.05$). Results are presented as mean ± SEM.

Post-morphine DHPG Challenge

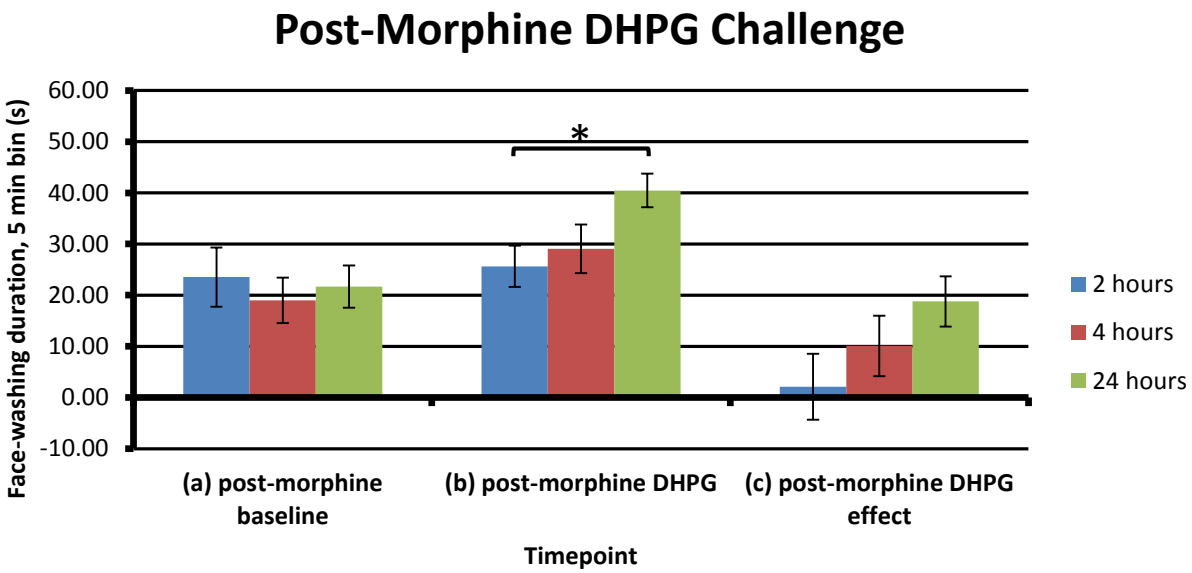

Figure 4 Post-morphine DHPG challenge This figure shows the average duration of face-washing behavior, in seconds, during the final five minutes of the baseline recording (a) and first five minutes of the post-DHPG injection recording (b), by group following six days of morphine administration. (c) shows the average post-morphine DHPG effect by group, defined as the difference between (b) and (a). A significant difference between the 2 and 24 hour groups was seen at the post-DHPG injection timepoint following repeated morphine administration. (*) indicates a significant difference ($p < 0.05$). Results are presented as mean ± SEM.

Baseline Face-Washing Activity

The average duration (in seconds) of face-washing behavior in the final 5 minutes of the post-morphine baseline recording is presented by group (2, 4, or 24 hours following the final

morphine injection) in Figure 4a. One-way ANOVA showed no significant differences between the experimental groups ($F(2,27) = 0.204$, $p = 0.816$). The post-morphine baseline face-washing levels were not significantly different from those seen in the pre-morphine recordings as shown by paired t-test ($t(29) = 1.451$, $p = 0.158$).

DHPG-Induced Face-Washing Activity

The average duration of face-washing behavior (in seconds) in the 5 minutes immediately following intrastriatal DHPG injection is presented by experimental group in Figure 4b. One-way ANOVA showed a significant difference between the experimental groups ($F(2,27) = 4.497$, $p < .05$). Tukey's post hoc comparison revealed a significantly lower duration of face-washing behavior in response to DHPG administration at 2 hours post-morphine administration as compared to 24 hours post-morphine administration ($p < 0.05$).

DHPG Effect

The difference in face-washing behavior duration (in seconds) between the post-DHPG and the baseline at the different time-points following morphine administration are presented in Figure 4c. One-way ANOVA revealed no significant differences between the groups ($F(2,27) = 2.427$, $p = 0.107$). However, post-hoc comparison revealed a trend for a reduction in the effect of DHPG to increase face-washing behavior at 2 hours following the last morphine administration. For that group, a strong trend toward reduction in DHPG-induced increased face-washing was observed

when comparing baseline and post-DHPG injection face-washing behavior following repeated morphine administration ($t(8) = 2.139$, $p = 0.065$).

ΔDHPG Effect

The baseline durations of face-washing and the durations of face-washing post-DHPG injection both before and after morphine administration for each of the experimental groups are presented in Figure 5. The differences in DHPG-induced increase in face-washing behavior between the post- and pre-morphine administration (i.e. ΔDHPG effect = post-morphine DHPG effect minus pre-morphine DHPG effect) are presented for each experimental group in Figure 6. One-way ANOVA revealed no significant differences between the experimental groups in this ΔDHPG effect ($F(2,27) = 3.272$, $p = 0.053$). However, using a paired t-test, a significant difference was observed between the pre- and post-morphine DHPG effects at 2 hours following the last morphine administration (Figure 5, $t(8) = 2.892$, $p < 0.05$).

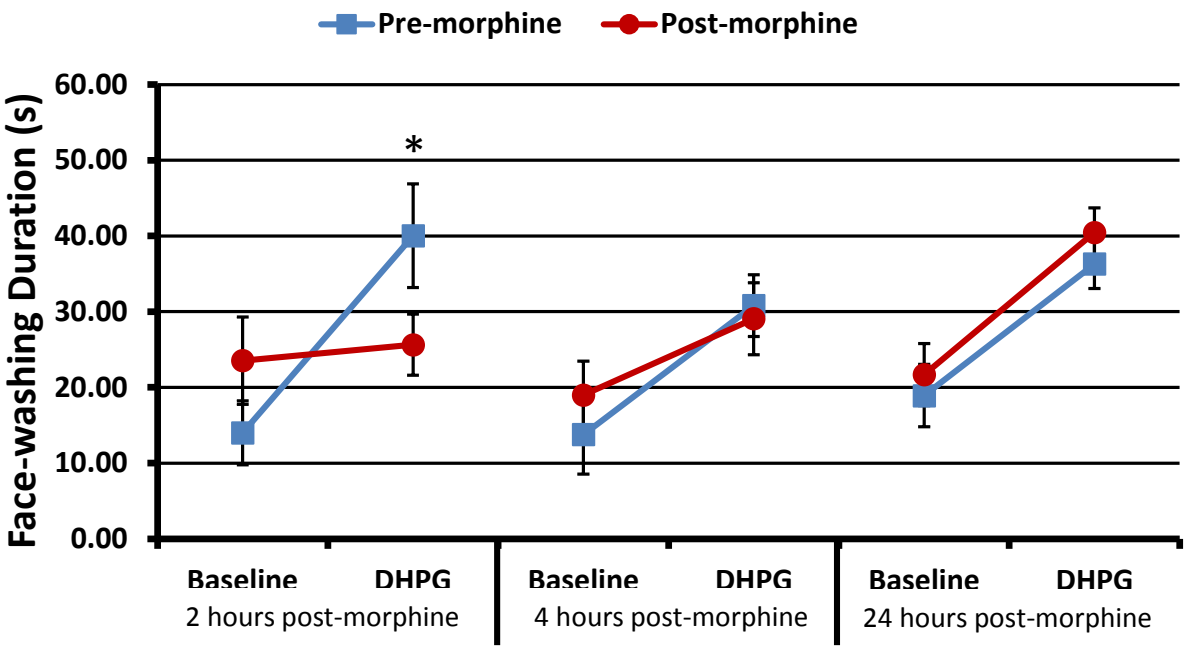

Figure 5 Level of face-washing behavior by timepoint and group This graph presents the baseline durations of face-washing and the durations of face-washing post-DHPG injection both before and after morphine administration for each of the experimental groups. A significant difference between the pre- and post-morphine responses to DHPG was observed 2 hours following the last morphine administration. (*) indicates a significant difference ($p < 0.05$). Results are presented as mean ± SEM.

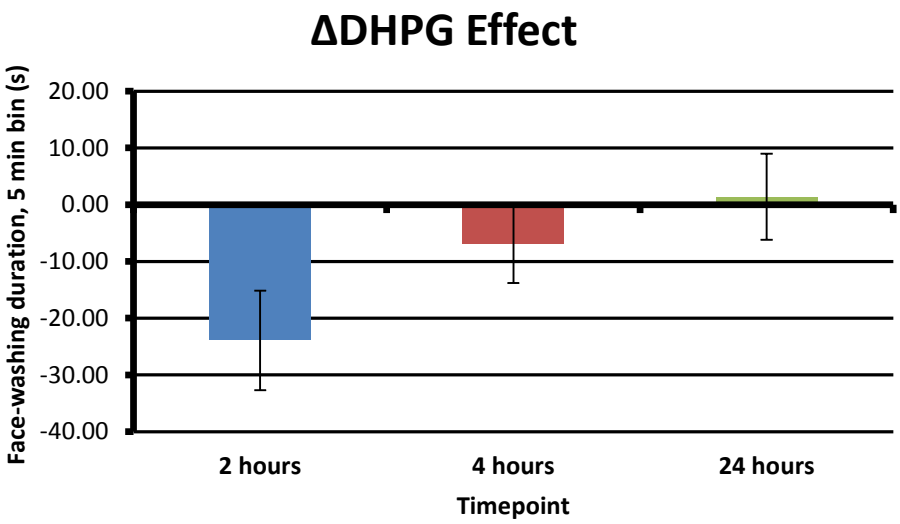

Figure 6 ΔDHPG effect This figure presents for each experimental group the ΔDHPG effect, defined as the post-morphine DHPG effect minus the pre-morphine DHPG effect. Results are presented as mean ± SEM.

CHAPTER IV

DISCUSSION

Interpretation of Results

In this study, a significant increase in face-washing behavior was observed in drug-naïve animals following microinjection of a low dose (10 nmol) of DHPG into the dorsal striatum. This is in line with the previous work demonstrating DHPG-induced increase in face-washing behaviors when administered ICV (Barton and Shannon, 2005; Hikichi et al., 2008). Importantly, no differences were observed between the experimental groups, either at baseline or post-DHPG. Thus, differences between the various groups observed following repeated morphine administration are not due to pre-existing differences prior to the morphine administration.

Morphine administration did not alter baseline face-washing behaviors in any of the experimental groups. Additionally, there were no significant differences in baseline face-washing behaviors between the various experimental groups following the repeated morphine administration. Thus, morphine per se and the withdrawal from morphine have no direct effects on face-washing behavior. Accordingly, differences in DHPG-induced face-washing behaviors between post- and pre-morphine administration are likely due to the effects of morphine administration on mGluR1 activity rather than locomotor activity or stereotyped behaviors in general.

Notably, this study demonstrated that repeated exposure to morphine does have an effect on the DHPG-induced increase in face-washing behaviors. Two hours following the final administration of morphine, a reduced effect of DHPG on face-washing behaviors was observed. In this group, the ΔDHPG effect was well below zero, indicating a decrease in the previously seen increase in face-washing behavior induced by DHPG (i.e. microinjection of DHPG into the dorsal striatum did not cause a significant increase in face-washing behavior as compared to the effect observed prior to morphine administration). No significant effect on DHPG-induced increase in face-washing behaviors was observed at 4 and 24 hours following the final administration of morphine. In these groups, the ΔDHPG effect was approximately zero, demonstrating the similarity of the pre- and post-morphine DHPG challenge results (i.e. microinjection of DHPG into the dorsal striatum caused an increase in face-washing behavior similar to that seen prior to morphine administration).

The marked decrease in the response to a DHPG challenge 2 hours following the final administration of morphine suggests that repeated administration of morphine causes a decrease in mGluR1 activity in the dorsal striatum of adolescent mice. This study also suggests that this is a direct effect of morphine, and not withdrawal, given that the effect was observed only 2 hours, but not 4 and 24 hours, following the last morphine administration. This is consistent with morphine's half-life in adolescent mice (i.e. approximately 24 minutes, Diaz, et al., 2007) as well as morphine activating and antinociceptive effects that are also known to last for approximately two hours in mice (Eitan, et al., 2003).

Importance of Results

Our previous study demonstrated that repeated morphine administration alter the responses of the D2-like dopamine receptors in adolescent mice (Hofford, et al., 2012). The current study demonstrated that repeated exposure to morphine also modulates mGluR1 activity in the dorsal striatum of adolescent mice. This is significant because it contributes to a more complete understanding of the factors that contribute to development of opioid addiction during adolescence. Future studies should examine whether this decrease in mGluR1 activity following repeated administration of morphine is age dependent, and to what extent repeated morphine administration will alter mGluR1 activity in adults. This will aid in determining the contribution of altered mGluR1 activity to the increased susceptibility of adolescents to drugs of abuse. Identifying age-specific factors that contribute to increased susceptibility for opioid abuse has the potential to reveal new targets for pharmacological manipulations for the treatment of opioid-abusing adolescents. Furthermore, it is likely that overlap exists in the mechanisms that contribute to the addictive potential of opioids and other drugs of abuse. Thus, the knowledge acquired in this study could be used to facilitate broader understanding of the factors that contribute to development of addiction in adolescents in general.

Limitations of the Study

The major limitation of the study is the small sample sizes for some of the groups. The sample size should be increased in future studies. The lack of significance in the ΔDHPG effect could be due to these small sample sizes. Additionally, this study employed scoring of video recordings by observers blind to the experimental conditions. We observed a relatively large variability

between the scores of various observers. This variability is magnified by the large variability in baseline face-washing behaviors between animals. Increasing inter-rater reliability in future studies would lead to smaller standard errors and possibly lead to clearer distinctions between groups.

Future Studies

This study was the first phase of a larger project examining the overall effect of repeated opioid administration on mGluR activity in adult and adolescent mice, and the differences in this effect between the two groups. Short-term effects of repeated morphine administration on mGluR1 signaling in the dorsal striatum of adolescent mice were examined behaviorally.

Future studies will examine the long-term behavioral effects of morphine exposure on the mGluR system in adolescent mice, as well as the short- and long-term effects on adult mice. The effects of other opioids on the mGluR system also need to be studied, since previous research has suggested that some opioids may cause less damage to neurotransmitter signaling than others, as mentioned in Chapter I. Additionally, the long-term implications of this alteration in mGluR signaling will be examined. Molecular changes in mGluR signaling will be examined in both age groups as well, looking at both short- and long-term changes in receptor activity.

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